UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte JEFFREY R. SAMPSON

Appeal No. 2006-3289
Application No. 09/358,141

ON BRIEF

Before GRIMES, GREEN, and LEBOVITZ, Administrative Patent Judges.

LEBOVITZ, Administrative Patent Judge.

DECISION ON APPEAL

This appeal involves claims to methods of synthesizing unstructured nucleic acids. The Examiner has rejected the claims as obvious. We have jurisdiction under 35 U.S.C. § 134. We affirm.

Background

"Naturally occurring ribonucleic acid (RNA) and deoxyribonucleic (DNA) acid molecules contain bases which are capable of forming base pairs through hydrogen bond interactions with complementary bases." Specification, page 1, lines 5-7. Base pairing between complementary nucleotides in a single molecule of DNA or RNA (intramolecular) results in the formation of secondary structures, such as loops,

duplexes, hairpin structures, three-way junctions, and four-way junctions. Id., page 1, lines 17-25. Secondary structure in a nucleic acid molecule can interfere with hybridization of complementary nucleic acid molecules, limiting "the sensitivity, specificity and utility of applications that rely upon hybridization of probes to analyze nucleic acid molecules." Id., page 2, lines 1-3. These applications include in situ hybridization, FISH, DNA array technology, and gene expression assays. Id., page 2, lines 3-7. The problem of target secondary structure is a particular problem with methods, such as polymerase extension and oligonucleotide ligation assays, which use short probes that "are less effective in competing with the intramolecular base-pairing interactions." Id., page 2, lines 8-13. The instant application provides nucleic acid molecules that contain modified nucleotide bases which do not form stable base hydrogen bonds with each other, resulting in reduced levels of secondary structure. Id., page 3, lines 20-24; page 4, lines 3-6 and 19-23. Fig. 1 shows examples of natural and modified nucleotides, and their ability to form stable base pairs.

Discussion

Claim construction

Claims 1 and 25-35, which are all the pending claims, are on appeal. Brief, page 2. Claim 1 is the only independent claim on appeal. Appellant states that dependent claims 25-35 "recite further features and/or combinations of features . . . that are patentably distinct from the prior art of record," but did not provide specific reasons for their patentability. Id., page 9. "A statement which merely points out what a claim

recites will not be considered an argument for separate patentability of the claim." 37 C.F.R § 41.37(c)(1)(vii). Consequently, dependent claims 25-35 stand or fall together with claim 1.

Claim 1 reads as follows:

> 1. A method of synthesizing an unstructured nucleic acid, the method comprising steps of:
>
> providing a nucleic acid template strand including a first template sequence element and a second template sequence element that is substantially complementary to the first template sequence element;
>
> providing a collection of nucleotides sufficient to synthesize a nucleic acid strand complementary to at least a portion of the template nucleic acid strand, which portion includes the first and second template sequence elements, the collection including at least a first complementary nucleotide that hybridizes with a first residue within the first sequence element on the template strand and a second complementary nucleotide that hybridizes with a second residue within the second sequence element on the template strand, wherein the first and second residues are complementary to one another but the first and second nucleotides have a reduced ability to form a stable hydrogen bonded base pair; and
>
> contacting the template and the nucleotides with an RNA polymerase enzyme characterized by an ability to polymerize the nucleotides under conditions and for a time sufficient to synthesize an unstructured nucleic acid in which said first complementary nucleotide and said second complementary nucleotide of the unstructured nucleic acid do not form an intramolecular base pair.

The preamble of claim 1 states that the method is for "synthesizing an unstructured nucleic acid." According to the specification, secondary structure occurs in a single molecule of nucleic acid (i.e., single-stranded) when "base pairs . . . form between two regions within a single molecule of DNA or RNA (intramolecular) where the two regions contain [complementary] sequences that permit the formation of base pairs." Specification, page 1, lines 17-19. "The present invention provides a system for

the production of nucleic acid molecules with reduced levels of secondary structure." Id., page 3, lines 20-21. When the claims are read in view of the written description, the skilled worker would understand "unstructured nucleic acid" to mean that the nucleic acid has reduced levels of secondary structure. This is also consistent with the claim language which states that the first and second complementary nucleotides present in the unstructured nucleic acid "do not form an intramolecular base pair," which is the source of secondary structure (see above). Accordingly, we construe the claimed method as being for the synthesis of a single-stranded nucleic acid (e.g., DNA or RNA) that lacks secondary structure.

Claim 1 has three steps: 1) providing a nucleic acid template strand; 2) providing a collection of nucleotides sufficient to synthesize a complement of the template strand; and 3) contacting the template and nucleotides with an RNA polymerase under conditions to synthesize an unstructured nucleic acid.

The "nucleic acid template strand" includes "a first template sequence element and a second template sequence element that is substantially complementary to the first template sequence element." A template would be understood by the skilled worker as a sequence which is copied to produce the unstructured single-stranded nucleic acid. Darnell,[1] 104 ("The DNA from which the new strand is copied is called a template."). The claim does not expressly state whether the template strand is single- or double-stranded. However, because the strand serves as a template for RNA polymerase to produce an unstructured single-stranded nucleic acid, the most logical reading of the claim is to require the strand to also be a single-stranded template. This

[1] Darnell et al. (Darnell), Molecular Cell Biology, p. 104-05, 1994

construction does not exclude a double-stranded nucleic acid from being used in the claimed method as the source of the single-stranded template.

The first and second sequence elements are "substantially complementary" to each other. According to the specification, "two sequence elements are considered substantially complementary if at least 50% of the nucleotides in the two elements can form stable hydrogen bonds." Id., page 12, lines 2-4. "A sequence element is defined as part or all of a polynucleotide molecule consisting of at least one nucleotide." Id., page 11, lines 24-25. In view of this description, a first sequence element having two nucleotides would be "substantially complementary" to a second sequence element having two nucleotides if only one nucleotide (i.e., 50%) in each element was capable of forming a stable base pair with one another. Stable base pairs are formed "through hydrogen bond interactions with complementary bases." Id., page 1, lines 5-13. These include the naturally-occurring base pairs (A/T, A/U, and G/C), but also base pairs between naturally-occurring and modified bases. See e.g., id., page 23, line 7-page 29, line 3.

In the second step of the claimed method, a nucleotide collection is provided which is sufficient to synthesize a complement to the nucleic acid template strand. The collection includes at least "first" and "second" complementary nucleotides which "have a reduced ability" to base pair with one another, but are able to hybridize with their complementary nucleotides in the template strand. As explained in the specification, the "first" and "second" complementary nucleotides are typically nucleotide analogs derived from complementary bases, which are unable to base pair with each other, but retain the ability to base pair with their naturally occurring complement. For example, T

can base pair with 2-aminoA (an adenosine analog) and A can base pair with 2-thioT (a thymidine analog), but 2-aminoA and 2-thioT are unable to form a stable base pair. Id., Fig. 1.

The last step of the claimed method involves synthesis of the unstructured nucleic acid by contacting the template strand with the nucleotides and RNA polymerase.

Anticipation under 35 U.S.C. § 102

Vivekananda[2]

Claims 1 and 25-34 stand rejected under 35 U.S.C. § 102(e) as anticipated by Vivekananda.

Vivekananda teaches "nucleic acid binding ligands capable of binding to, identifying and/or neutralizing anthrax." Vivekananda, column 2, lines 20-24. "The meaning of 'nucleic acid ligand' specifically excludes nucleic acids that bind to another nucleic acid through a mechanism which predominantly depends on Watson/Crick base pairing." Id., column 8, lines 4-7. In its examples, Vivekananda describes using a prior art process known as SELEX to select nucleic acid ligands (also called "aptamers") which bind to anthrax spores. Id., column 37, lines 64-column 38, line 12 ("Example 1"). The nucleic acid ligands can be produced by enzyme amplification, including PCR. Id., column 20, lines 23-26. Naturally occurring and synthetic nucleotides can be incorporated into the ligands. Id., column 20, line 33-column 21, line 41.

[2] Vivekananda et al. (Vivekananda), U.S. Patent No. 6,569,630 B1, May 27, 2003

The Examiner states:

> Vivekananda teaches the instantly claimed methods of synthesizing nucleic acid polynucleotides with reduced secondary structure by incorporating the claimed modified bases, producing nucleic acid strands with a reduced ability to form intra-molecular base pairs (see especially col. 2, lines 20-65; col. 3, line 44-col. 5, line 67; col. 6, line 62-col. 10, line 31), comprising the polymerization of nucleotide precursors from a DNA or RNA template by an appropriate polymerase or transcriptase (see especially col. 22, line 29-col. 24, line 45), and which nucleotide precursors include 2-aminodeoxyadenosine 5'-triphosphate, 2-thiodeoxythymidine 5'-triphosphate, and inosine triphosphate (see especially col. 20, line 14-col. 22, line 9), whereby a nucleic acid molecule with reduced levels of cross-hybridization is synthesized.

Answer, page 4.

Appellant argues that Vivekananda "simply provides a laundry list of various modified nucleotide bases, but still does not provide for the step of claim 1" in which a collection of nucleotides is provided that includes "at least a first complementary nucleotide that hybridizes with a first residue . . . and a second complementary nucleotide that hybridizes with a second residue . . . wherein the first and second residues are complementary to one another but . . . have a reduced ability to form a stable hydrogen bonded base pair." Brief, page 4; claim 1.

To anticipate, every element and limitation of the claimed invention must be found in a single prior art reference, arranged as in the claim. Karsten Mfg. Corp. v. Cleveland Golf Co., 242 F.3d 1376, 1383, 58 USPQ2d 1286, 1291 (Fed. Cir. 2001).

In this case, we agree with Appellant that Vivekananda does not describe every element and limitation of the claimed subject matter. Beginning at column 20, line 33, Vivekananda generally describes nucleotide mimics and derivatives that may be incorporated into its nucleic acid ligand. However, claim 1 requires that a pair of

complementary nucleotides having "a reduced ability to form a stable hydrogen bonded base pair" be utilized as substrates in "synthesizing an unstructured nucleic acid." We have construed these complementary nucleotides to include nucleotide analogs capable of base pairing with their naturally-occurring complement, but not with each other. While Vivekananda lists complementary nucleotide analogs which would have been known by the skilled worker not to form stable base pairs, it doesn't teach the skilled worker to pick two complementary nucleotide analogs from the list that meet the claimed requirement and to use them in an enzymatic method of synthesizing a nucleic acid. A broad disclosure does not necessarily render obvious any species that happens to fall within it. In re Baird, 16 F.3d 380, 382, 29 USPQ2d 1550, 1552 (Fed. Cir. 1994).

An anticipating reference "must describe the applicant's claimed invention sufficiently to have placed a person of ordinary skill in the field of the invention in possession of it." In re Spada, 911 F.2d 705, 708, 15 USPQ2d 1655, 1657 (Fed. Cir. 1990). The Examiner has only pointed to broad, general disclosure, but has not explained how this disclosure would have placed a person of ordinary skill in the art in possession of the claimed invention, particularly the step in which two complementary nucleotides which "do not form an intramolecular base pair" are used in synthesizing an unstructured nucleic acid molecule. Accordingly, we conclude that the Examiner has not set forth adequate evidence to establish a case of prima facie anticipation. This rejection is reversed.

Kutyavin

Claims 1 and 25-35 stand rejected under 35 U.S.C. § 102(e) as anticipated by Kutyavin.[3]

Kutyavin describes a matched pair of oligonucleotides (ODNs) which are termed "Selective Binding Complementary (SBC) ODNs." Kutyavin, column 1, lines 64-65.

> [E]ach member of the pair is complementary or substantially complementary in the Watson Crick sense to a target duplex sequence. However the ODNs include modified bases of such nature that the modified base forms stable hydrogen bonded base pairs with the natural partner base, but does not form stable hydrogen bonded base pairs with its modified partner. Generally speaking, this is accomplished when in a hybridized structure the modified base is capable of forming two or more hydrogen bonds with its natural complementary base, but only one or no hydrogen bonds with its modified partner. Thus, the matched pair of oligonucleotides in accordance with the present invention do not form substantially stable hydrogen bonded hybrids with one another.

Id., column 1, lines 40-53.

The Examiner states:

> The procedure disclosed by Kutyavin ... is indistinguishable from the instantly claimed methods because both methods involve the synthesis of oligonucleotides in the presence of modified bases, to produce nucleic acids with reduced ability to hybridize to complementary bases by reducing the ability to form Watson-Crick base pairing. The characteristic of reduced ability to hybridize to complementary, modified bases exists in intra-molecular as well as inter-molecular complementary nucleic acid strands.

Answer, page 5.

Appellant argues that "Kutyavin discloses double strand invasion with two strands of the SBC ODNs. Thus, the feature of claim 1 of a single-stranded unstructured nucleic acid is not taught or suggested by Kutyavin." Reply brief, page 8. Appellant also asserts that the claimed method results in polymerization of a nucleic

[3] Kutyavin, U.S. Patent No. 5,912,340, June 15, 1999

acid which comprises nucleotides that do not form intramolecular bonds, which is novel and unobvious in view of Kutyavin. Brief, page 8. Appellant argues that "simply providing a target with the desired characteristics does not anticipate a method of synthesizing the unstructured nucleic acid by the steps of claim 1." Id., page 9.

Contrary to Appellant's assertions, we find that Kutyavin does anticipate the claimed subject matter by teaching every element and limitation recited in it. Kutyavin expressly states that "nucleotides may be incorporated either enzymatically or via chemical synthesis" to form SBC oligonucleotides. Kutyavin, column 14, lines 35-45. Claim 1 is a method of synthesizing a nucleic acid strand with an RNA polymerase, which is an enzyme. A species (i.e., enzymatic synthesis) which is specifically disclosed in a prior art reference is anticipatory even though it appears "without special emphasis in a longer list." Perricone v. Medicis Pharm. Corp., 432 F.3d 1368, 1376, 77 USPQ2d 1321, 1326 (Fed. Cir. 2005). Consequently, we find that Kuyavin teaches the enzymatic synthesis of a nucleic acid as recited in claim 1.

Claim 1 also requires "contacting the template and the nucleotides with an RNA polymerase enzyme." Kutyavin does not disclose an RNA polymerase, but it does describe a process of synthesizing DNA enzymatically by nick-translation using a nucleic acid template (column 28, line 23: "pHPV-16"), DNA polymerase (column 28, line 22), and an adenine nucleotide analog precursor (column 28, line 38). It also discloses that the SBC oligonucleotides can be RNA. Id., column 5, lines 30-51, particularly, lines 49-50 in which the nucleotide analogs are ribonucleotides ("R is ... OR_2, where R_2 is . . . H in case of RNA"). Ribonucleotides are substrates for RNA polymerase, but not DNA polymerase. Darnell, 104-105, particular Fig 3-22. The

skilled worker would have recognized that, when ribonucleotides (Kutyavin, column 5, lines 49-50) are utilized to enzymatically synthesize an oligonucleotide (Kutyavin, column 14, lines 35-45), RNA polymerase would be required.

The prior art may anticipate the claimed subject matter when the claimed limitations are not expressly found in the reference, but are inherent to it. Inherency inquires whether a subject matter is "necessarily" present in the prior art reference. "Inherent anticipation requires that the missing descriptive material is 'necessarily present,' not merely probably or possibly present, in the prior art." Trintec Indus. v. Top-U.S.A., 295 F.3d 1292, 1295, 63 USPQ2d 1597, 1599 (Fed. Cir. 2002). We find this to be the case here. As indicated above, Kutyavin discloses that the SBC oligonucleotides can be comprised of ribonucleotides. When enzymatic synthesis is utilized to prepare an SBC comprising ribonucleotides, the use of RNA polymerase, rather than DNA polymerase, is necessary because an RNA polymerase is the appropriate enzyme for incorporating ribonucleotides into the strand. Darnell, 104-105. Consequently, producing an oligonucleotide with ribonucleotides necessarily would involve the step of contacting the template with RNA polymerase, resulting in a process which carries out the nucleotide "providing" and template "contacting" steps of claim 1.

Claim 1 also requires a first step of "providing a nucleic acid template strand including a first template sequence element and a second template sequence element that is substantially complementary to the first template sequence element." According to the Examiner (Answer, page 11), a nucleic acid template strand is described by Kutyavin at column 4, lines 52-64:

> As it is described in more detail below, an important use of the SBC ODNs of the present invention is hybridization with secondary structure of mRNA wherein the mRNA itself forms a duplex, such as in hairpin loops. It is known that secondary structure of mRNA and ribosomal RNA do not have two strands in the strict sense of that term. Nevertheless, unless the context otherwise indicates, in the present description the terminology "two strands" of double stranded nucleic acids also refers to the two complementary portions of duplex mRNA or of duplex ribosomal RNA as well. The general concept of double stranded DNA and of secondary structure in mRNA and ribosomal RNA is covered in this description by the term "duplex nucleic acid".

Appellant asserts that "[i]n this passage, Kutyavin is referring to the formation of probes in, for example, a hybridization assay. In contrast, as recited in claim 1, the targets themselves are synthesized." Brief, page 8.

We do not find Appellant's argument persuasive. Kutyavin expressly refers to "two complementary portions of duplex mRNA." The complementary portions, which are present in the same nucleic acid, form a duplex hairpin loop because their complementarity permits the portions to hybridize together. Kutyavin describes probes to the region of mRNA comprising the complementary regions. Appellant has overlooked the fact that Kutyavin also enables the person of ordinary skill in the art to make the probes enzymatically using an RNA polymerase as claimed. A template for making the probes would have both complementary regions, meeting the claimed limitation of "a first template sequence element and a second template sequence element that is substantially complementary to the first template sequence element." Appellant has not distinguished the claimed template from this disclosure.

Furthermore, as we have construed the claim, a sequence element can contain as few as two nucleotides. SEQ ID NO:1 of Kutyavin discloses a first element of 4

nucleotides which contains 2 nucleotides which are complementary to 2 nucleotides (i.e., 50%) within a second element of 4 nucleotides (**CT**GACAACGA TCGG**AG**GACC GAAGGAGC) (elements are underlined; complementary nucleotides are in bold). Substantial complementarity is met "if at least 50% of the nucleotides in the two elements can form stable hydrogen bonds." Specification, page 12. A nucleic acid template which comprises SEQ ID NO:1 (or its complement) would therefore meet the claimed requirement.

Appellant argues that Kutyavin in his examples describes a pair of nucleic acid molecules, not "an unstructured single-stranded nucleic acid molecule" which is recited in claim 1. Reply brief, page 7. We do not find this argument persuasive. Although Kutyavin describes matched pairs of oligonucleotides, each member of the pair would be synthesized by a process which meets all the limitations required by claim 1. This process is discussed above. The claim does not exclude synthesizing both members in the same reaction mixture at the same time. Appellant has not distinguished Kutyavin's method of synthesizing nucleic acid from his own.

Appellant also contends that "Kutyavin discloses double strand invasion with two strands of the SBC ODNs. Thus, the feature of claim 1 of a single-stranded unstructured nucleic acid is not taught or suggested by Kutyavin." Reply brief, page 8. Appellant is focusing on a method of use described by Kutyavin for the SBC oligonucleotides, while ignoring the fact that Kutyavin also describes unstructured nucleic acids and how to prepare them. In Table 2, for example, Kutyavin provides the sequence of two probes, each comprised of non-naturally occurring nucleotide analogs which have a reduced ability to form stable hydrogen bonds with each other (as

required by claim 1), but which base pair with their naturally occurring complement. Kutyavin, columns 23-24, Table 2, SBC(V) and SBC(VI). See also Answer, page 10. We find that the property of having a reduced ability to form stable intramolecular bonds (and hence an unstructured nucleic acid) is an inherent characteristic of Kutyavin's SBC probes which contain nucleotide analogs that do not form stable base pairs with one another. Scheme C provided with the Brief illustrates the reduced ability to form an intramolecular hairpin structure when nucleotide analogs are incorporated into a nucleic acid. This structure would also occur in the oligonucleotides described by Kutyavin. For example, in Tables 1 and 2 (columns 23-24), oligonucleotides are shown in which the complementary pair dG and dC are replaced by nucleotide analogs dI and dP (column 23, lines 30-32), and in which the complementary pair dA and dT are substituted by nucleotides analogs d2amA and d2sT (column 23, lines 25-26). See also Answer, page 10. Each of the complementary analogs has an impaired ability to base pair with its modified complement, but not with its naturally-occurring complement. Thus, an oligonucleotide of Kutyavin has the same appearance and properties as the nucleic acid in Scheme C, where naturally-occurring nucleotides are substituted by analogs unable to form stable hydrogen bonds with one another.

"An anticipating reference must describe ... [the claimed] ... subject matter with sufficient clarity and detail to establish that the subject matter existed in the prior art and that such existence would be recognized by persons of ordinary skill in the field of the invention." Crown Operations Int'l, Ltd. v. Solutia Inc., 289 F.3d 1367, 1375, 62 USPQ2d 1917, 1921 (Fed. Cir. 2002). For the foregoing reasons, we conclude that method recited in claim 1 is described with sufficient detail clarity to establish its

existence in Kutyavin and that its existence would have been recognized by persons of ordinary skill in the art. Claims 25-35 fall with claim 1 since separate reasons for patentability were not provided. This rejection is affirmed.

Other Issues

If prosecution in this application is continued, the Examiner should consider whether the RNA polymerase recited in claim 1 would be capable of incorporating deoxyribonucleotides as recited in dependent claims 25, 26, 27, 28, and any others.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 CFR § 1.136(a).

AFFIRMED

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